

Substitute the Sequence Listing on pages 37-49 with the amended Sequence Listing pages 1-13 submitted herewith.

REMARKS

The Amendments

Applicants have amended the specification by removing the Sequence Listing on pages 37-49 and adding amended Sequence Listing pages 1-13. The substitute Sequence Listing is the same as the Sequence Listing as filed, except that the information relating to the applicants, docket number, current application number, filing date, and priority information has been revised in accordance with United States Patent practice and the status of this application. Applicants have also amended the specification to correct a typographical error in the sequence of SEQ ID NO:5 recited on page 24, line 15 and page 26, line 20. Specifically, the sequence of SEQ ID NO:5 should contain a “Q” instead of an “O.” Support for the amendments can be found in the Sequence Listing as originally filed (page 47 of the specification). These amendments include no new matter.

The Sequence Listing Requirements

The Notice states that the application does not contain a statement under 37 C.F.R. §1.821(e), §1.821(f), §1.821(g), §1.825(b), or §1.825(d). The Notice also states that a computer readable form copy of the Sequence Listing has not been submitted as required under 37 C.F.R. §1.821(e). As discussed above, applicants have amended the Sequence Listing. Accordingly, applicants submit a paper copy of the amended Sequence listing, a computer



Appendix

On page 24, lines 6-21 of the specification:

After the surgical removal of spleens from naive C57BL/6 mice, the spleens were crushed through a sterile grid directly into a dish containing DMEM medium supplemented with 2% FCS. The cells were vigorously suspended, transferred into Falcon tubes, and centrifuged for a few seconds after which the tubes were let stand for 10 minutes. Single spleen cells remained in the supernatant, which was poured off into another tube. The leukocytes were counted by trypan-blue exclusion and adjusted to a cell density of 7x10⁶ viable cells per ml. All three peptides used for pulsing (peptide 1 [KTWGOYWQV] KTWGQYWQV (SEQ ID NO:5), peptide 2 ITDQVPFSV (SEQ ID NO:6) and peptide 3 VLYRYGSFSV (SEQ ID NO:7)) were purchased from MWG Biotech (Ebersberg, Germany). A 100-fold peptide stock solution in PBS was prepared using several sonification steps to promote dissolution. The peptide solution (10 nM) was added to the spleen cell suspension and incubated for 1 hour at room temperature and for another 1 hour at 37°C under 5%CO₂. The peptide-pulsed spleen cells were injected intraperitoneally into the mice (2x10⁷ cells per mouse).

On page 26, lines 1-22 of the specification:

Five different immunogenic peptides from the hgp100 protein have been identified previously and shown to be reactive with different hgp100 TILs (Kawakami et al., J. Immunother. 21 (1998), 237-246). Two of the nonamer peptides which were also used in the present application, peptide 1 G9(154-162) and peptide 2 G9(209-217), appeared to be immunodominant and were widely recognized by TILs (Pass et al., Cancer J. Sci. Am. 4 (1998), 316-323; Rivoltini et al., J.

Immunol. 156 (1996), 3882-3891; Salgaller et al., Cancer Res. 56 (1996), 4749-4757; Clay et al., J. Immunol. 162 (1999), 1749-1755). Peptide 3, the decamer G10(476-485), is expressed on the surface of melanoma cells and is able to induce melanoma-reactive CTLs from peripheral blood lymphocytes (PBL) of melanoma patients by repeated in vitro stimulation (Salgaller et al., Cancer Res. 55 (1995), 4972-4979). Thus, these gp100 epitopes were considered as good candidates for use in peptide-based immunotherapies. Recently, treatment of melanoma patients with synthetic peptides representing putative CTL-specific epitopes summarized in a review of Rosenberg ((1997), loc. cit.) have shown some success when used for ex vivo pulsing of autologous APCs, for instance dendritic cells which are specialized for the induction of primary T cell response (Nestle et al., Nat. Med. 4 (1998), 328-332). Three of previously described CTL epitopes used for pulsing autologous spleen cells from C57BL/6 mice are indicated in the sequence alignment of human and mouse gp100 (Fig. 1B), peptide 1 ([KTWGOYWQV]
KTWGQYWQV; SEQ ID NO:5), peptide 2 (ITDQVPFSV; SEQ ID NO:6) and peptide 3 (VLYRYGSFSV; SEQ ID NO:7). Peptide 2 is identical between human and mouse, while the peptides 1 and 3 differ in one amino acid.

readable form copy of the amended Sequence listing and a Statement, in accordance with 37 C.F.R. §1.821 (c), (g) and (e). Therefore, the requirements for the Sequence Listing have been satisfied.

The Oath or Declaration

The Notice states that the oath or declaration is unsigned and that a surcharge for a small entity is due. Accordingly, applicants submit a check for \$65.00 to complete payment of the surcharge as set forth in 37 C.F.R. § 1.16 (e) for a small entity. Applicants also submit an executed Declaration and Power of Attorney signed by the inventors.

The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account No. 06-1075. A duplicate copy of this Response is enclosed herewith.

Respectfully submitted,



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